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TITLE: Hyperexcitability in Sensory Circuits in Fragile X Syndrome

PRINCIPAL INVESTIGATOR: Anis Contractor

CONTRACTING ORGANIZATION: Northwestern University
Chicago, IL 60611

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Northwestern University Chicago, IL 60611

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12. DISTRIBUTION / AVAILABILITY STATEMENT

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13. SUPPLEMENTARY NOTES

14. ABSTRACT: Fragile X syndrome (FXS) is the most common single gene cause of autism and intellectual dysfunction. It is marked by devastating alterations in cognition and behavior that originate in infancy. Approximately 1 in 4000 live births are affected by the disorder; therefore it represents a major health problem that also profoundly impacts a sizeable number of military families. A core symptom of the disorder is hypersensitivity of the senses, including hypersensitivity to touch, such that normal sensory stimuli are perceived as aversive. This contributes directly to many of the challenges faced by FXS individuals, including hyperarousal, social withdrawal and anxiety. The two partnering laboratories have collaborated on understanding this disruption for a number of years by working on an experimental mouse model of FXS. Studies from our laboratories have begun to define how the development of synapses and circuits in the sensory cortex are altered in FXS. We have found that there is abnormal activity in parts of the brain that process sensory inputs that could be due to changes in the neurotransmitter GABA, which normally dampens brain activity. In this proposal we will determine the extent of the alteration in synapses, neurons, circuits and behavior in the FXS model and ask the following three questions: 1) how do changes in the activity of neurons in the brain of FXS mice lead to an altered response to touch? 2) what are the alterations in GABA and brain connectivity that lead to a difference in the response of neurons in the circuit? 3) can we fix the problems in the aberrant response to touch in mice by improving GABA signaling during early brain development? These studies are designed to understand a critical problem in the FXS field, address important knowledge gaps, and ultimately to determine whether we can find ways to rectify the development of brain circuits that contribute to altered touch sensation. Our experimental design will employ cutting-edge techniques to record from neurons in the sensory cortex and is designed to incorporate the complementary expertise of the partnering laboratories. The ultimate outcome will be in identifying the network basis for hyperarousal to sensory stimuli, a hallmark symptom in FXS, and will inform the future development of novel treatments for children with FXS.

15. SUBJECT TERMS

None listed

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified	18	**19b. TELEPHONE NUMBER** *(include area code)*

TABLE OF CONTENTS

1. INTRODUCTION:

Fragile X syndrome (FXS) is the most common inherited form of intellectual disability and the largest genetically identified cause of autism affecting roughly 1 in 2,500 males. One of the core deficits in autism, which is particularly prominent in FXS individuals, is the problem of hypersensitivity to a variety of sensory stimuli, which results in hyperarousal, anxiety and seizures. The underlying alterations in the development of neuronal circuits that are the basis for sensory problems in autism are not well defined. In this project the multi-PI team proposed to understand the circuit basis for altered sensory responses in the mouse model of FXS. Both in vivo imaging of neuronal activity as well as in vitro recording of individual neurons is proposed to map the connectivity and functional changes in the somatosensory cortex focusing on the role of GABAergic neurons. Furthermore a strategy to alleviate these deficits by targeting the maturation of GABAergic interneurons will be employed.

2. KEYWORDS:

Autism, Fragile X, GABA, Interneuron, Sensory hypersensitivity, TrkB, Synapse

3. ACCOMPLISHMENTS:

The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.

What were the major goals of the project?

List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

Major Goals:

The Multi-PI proposal had three integrated aims. The SOW was divided so that Sp Aim 1 would be carried out in the Portera-Cailliau laboratory, Sp Aim 2 would be performed in the Contractor laboratory and Aim3 would be performed in both laboratories. For the first year of the award tasks in Aim 1 and Aim 2 were prioritized.

Aim1: To test whether dysfunctional inhibitory circuitry in barrel cortex causes the lack of neuronal adaptation and avoidance behaviors (tactile defensiveness) in *Fmr1* KO mice

a. Determine whether tactile disturbances also manifest in response to visual stimuli

b. Determine whether increased locomotor activity in Fmr1 mice in response to sensory stimuli is an avoidance response

c. Determine whether adaptation deficit is due to altered inhibition

d. Determine whether the sensory alterations result from loss of FMRP during critical period development

Aim 2: Determine the alteration in connectivity and function of synapses in the sensory microcircuit

a. Determine whether there are disruption in the fine grain connectivity of interneuron subtypes and principal neurons in layer IV of the somatosensory cortex of Fmr1 KO mice

b Determine whether there are alteration in the connectivity of layer II/III neurons in Fmr1 KO mice

c. Determine whether the development of extrinsic connectivity from thalamus is altered in Fmr1 KO mice

d. Determine whether the dynamic properties of individual synaptic connections in the somatosensory cortex are altered in FXS mice

During the first cycle of the award we have proposed to begin work on the objectives of these two aims. Advances in these are outlined below.

What was accomplished under these goals?
For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

Aim 1: To investigate the role of cortical inhibitory circuitry underlying the lack of neuronal and behavioral adaptation to repetitive whisker stimulation in *Fmr1* KO mice:

The major goal of Aim 1 was to test three separate but related hypotheses:

1. That the behavioral phenotype of *Fmr1* KO mice to repetitive sensory stimulation is an avoidance response to an aversive sensory stimulus.
2. That a defect in interneuron circuitry in the cortex is responsible for the lack of sensory adaptation in *Fmr1* KO mice.
3. That this major sensory processing defect in *Fmr1* KO mice depends on loss of FMRP prior to the critical period but persists into adulthood.

Major Task 1: To test whether dysfunctional inhibitory circuitry in barrel cortex causes the lack of neuronal adaptation and avoidance behaviors (tactile defensiveness) in Fmr1 KO mice:

Subtask 1: Does the behavioral manifestation extend to repetitive visual stimuli? (Months 1-12)
We proposed to investigate whether the neuronal adaptation to chronic sensory stimulation was also absent in primary visual cortex (V1) in *Fmr1* KO mice. We performed some pilot experiments in the first funding period, to determine the ideal parameter to evoke adaptation in V1 of control WT mice. So far, we find that even after a few epochs (3 s 'on', 4 s 'off') of stimulation using sinusoidal gratings drifting in the same direction, we see decrements in the magnitude of evoked responses in pyramidal neurons in V1 of WT mice. In the next funding cycle, we will complete the experiments, comparing WT and *Fmr1* KO mice.

This subtask will be completed in the second year of the grant.

Subtask 2: Do adult *Fmr1* KO mice also exhibit neuronal and behavioral adaption to repetitive whisker stimulation and do network alterations (loss of neuronal adaptation) require loss of FMRP before and up to the critical period? (Months 1-6)

In a series of studies that we published recently [1], we demonstrated that adult *Fmr1* KO mice perceive repetitive whisker stimulation as aversive, because they run preferentially away from the side of stimulation (Fig. 1). This was the first demonstration, to our knowledge, of an avoidance response in fragile X mice that is akin to tactile defensiveness in humans with FXS.

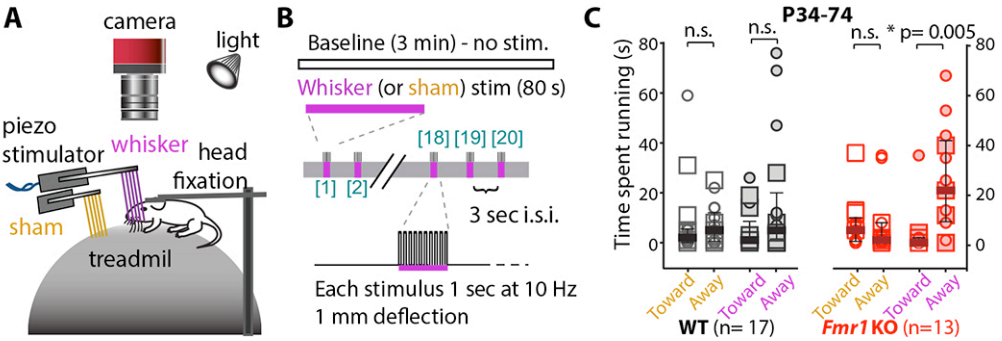

Figure 1: Avoidance behavior in adult *Fmr1* KO mice to repetitive whisker stimulation resembles tactile defensiveness in FXS. A) Cartoon of behavioral setup A whisker stimulator comb of flexible wires, moved by a piezoelectric actuator, was intercalated between whiskers on the left snout or placed away from the whiskers (sham). **B)** Experimental design & timeline. **C)** Adult *Fmr1* KO mice (but not WT mice) spent significantly more time running away from side of the whisker stimulation (Pairwise rank-based two-group comparisons with 10,000 resamples and Bonferroni correction). [from He CX et al., 2017]

In the same study, we demonstrated that adult Fmr1 KO mice also failed to exhibit neuronal; adaptation to repetitive whisker stimulation, just as they do at 2 weeks of age (Fig. 2).

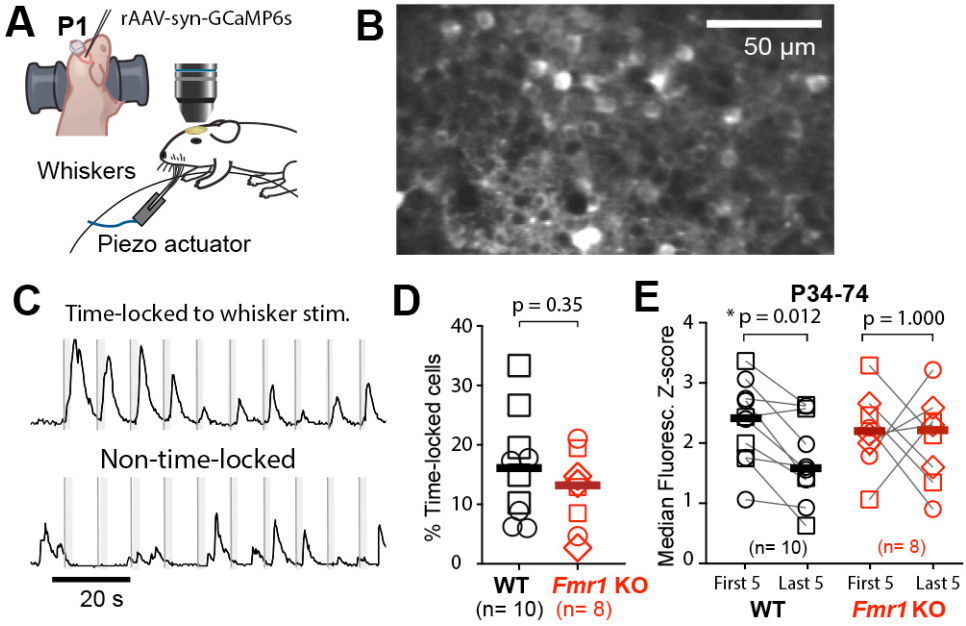

Figure 2: Lack of neuronal adaptation in adult *Fmr1* KO mice. A) rAAV-GCaMP6s was injected into S1 cortex at P1 or P20 (for imaging at 1-2 months of age). We confirmed targeting to barrel cortex with intrinsic signal imaging (not shown). A bundle of whiskers was stimulated. **B)** Field of view of L2/3 neurons expressing GCaMP6s at P15 (*xyt* projection of 100 consecutive frames, 7.8 Hz). **C)** Example fluorescence traces from L2/3 neurons with activity that is time-locked (*top*), or not (*bottom*), to epochs of whisker stimulation (light grey bars). **D)** The percentage of time-locked neurons was similar in WT *and* Fmr1 KO mice. Each diamond is median Z-score across all ROIs for a mouse. Bars are group medians. Two-group rank-based comparisons with 10,000 resamples, with Bonferroni correction. **E)** There was significant adaptation (decreasing Z-scores) for whisker-evoked activity across all in adult WT, but not *Fmr1* KO mice [From He CX et al., 2017]

We had also proposed to investigate whether FMRP is important for proper establishment of neuronal adaptation before the closure of the critical period in barrel cortex, by deleting Fmr1 in

cortical neurons after the 2nd postnatal week. We have obtained and crossed the necessary mouse lines: CamKII-Cre and lox-STOP-lox-Fmr1 conditional KO mice. Over the next funding period we will perform in vivo calcium imaging in these mice to test whether they also manifest a loss of neuronal adaptation to repetitive whisker stimulation (we predict that these cKO mice will have normal neuronal adaptation).

Subtask 3: Is the increase in locomotion/activity an avoidance response? (Months 12-36)

The goal will be to conduct simultaneous in vivo calcium imaging recordings in awake, head-restrained mice that are allowed to run on a floating polystyrene ball, so that both measures of sensory adaptation can be assessed and correlated in individual animals. We will be tracking locomotion and forelimb movements with a camera, as well as pupil diameter with a high-speed camera. As mentioned above (Subtask 2), we recently showed that adult *Fmr1* KO mice exhibit tactile defensiveness, an avoidance response to what is likely perceived as an aversive stimulus [1]. The goal now will be to test the hypothesis that FXS mice show signs of anxiety and stress, namely persistently dilated pupils (a manifestation of a hyperadrenergic, anxiety-like state). *Fmr1* KO mice with the least degree of neuronal adaptation (from calcium imaging) are expected to manifest the highest degree of anxiety (pupil dilation) and the least amount of behavioral habituation (changes in locomotion).

Subtask 4: Is the deficit in adaptation due to decreased inhibition? (Months 1-24)

The goal here was to search for a cellular mechanism of the loss of neuronal adaptation in *Fmr1* KO mice. Specifically, we proposed to test whether hypoactivity in either parvalbumin (PV) or somatostatin (SST) interneurons in barrel cortex could be to blame because of the known role of these inhibitory cells in silencing the activity of excitatory pyramidal neurons. These experiments will be conducted in Years 2 and 3 of the DoD grant. However, in parallel studies we have recently completed in our lab, we demonstrated that PV cells are indeed hypoactive in V1 of Fmr1 KO mice, a defect that correlates with poor behavioral performance on a perceptual learning task (Fig. 3).

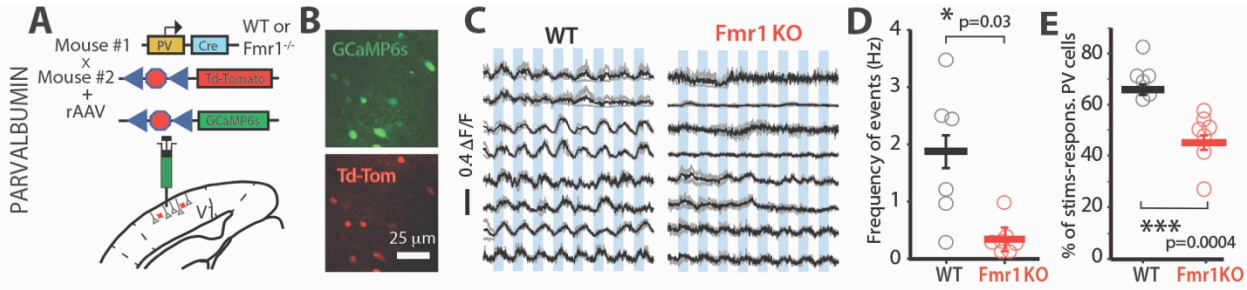

Figure 3: Alterations in orientation selectivity and tuning in V1 and reduced neuronal activity of PV neurons in *Fmr1*$^{-/-}$ mice. **A)** Strategy for selective GCaMP6s expression in PV interneurons. **B)** 2-photon images of PV neurons expressing GCaMP6s (green) and Td-Tom (red). **C)** Visually evoked GCaMP6s signals in PV cells were markedly reduced in *Fmr1*$^{-/-}$ mice. **D)** The frequency of visually evoked calcium transients in PV neurons is significantly lower in *Fmr1*$^{-/-}$ mice. **E)** The fraction of visually-responsive PV cells is significantly reduced in *Fmr1*$^{-/-}$ mice. We plan on using a similar approach in Subtask #4 of this proposal. (all Student t-tests; each symbol is a different mouse).

Aim 2: To determine the synaptic, cellular and local circuit basis for adaptation deficit in acute slices of somatosensory cortex

The major goal is to determine whether the connectivity and excitability of the major cell types in the somatosensory microcircuit is altered. Most of these experiments are ongoing but preliminary data suggest changes in synaptic connectivity during early development.

Major Task 2: Determine the alteration in connectivity and function of synapses in the sensory microcircuit

Subtask 1: Determine whether there are disruption in the fine grain connectivity of interneuron subtypes and principal neurons in layer IV of the somatosensory cortex of Fmr1 KO mice. Using single cell electrophysiological analysis the goal here is to determine whether the synaptic connections between major neuronal types in layer IV are altered. This is primarily done by recording from the principle neurons and different interneuronal types. In recently published data we had demonstrated that during early development synaptic input to PV interneurons is reduced during early development[2]

The first parameter that we have measured in the relative excitatory to inhibitory input to the principal neurons in Layer IV. Single cell patch clamp recordings were made from visually identified spiny stellate at two developmental timepoints. Monosynaptic inputs to Layer IV neurons were stimulated using an extracellular stimulating electrode. To record isolated EPSCs and IPSCs in the same cell without the use of pharmacological blockers, the ionic concentration of the intracellular solution was adjusted so that the reversal potential for AMPA receptors and GABA receptors was such that EPSCs could be recorded by holding the neuron at -60mV and IPSCs could be recorded by holding the neuron at 0mV (Figure 5). We found that there is a significant deficit in the monosynaptic inhibitory input to these neurons at early developmental stages in the Fmr1 KO mice (Fig 4). At P7 there was no IPSC detected in most cells whereas there was a robust EPSC (Fig 4A&C). This deficit persisted in the Fmr1 KO mice in recording from P9-10 mice. These data demonstrate that there is a large deficit in connectivity of layer IV neurons to local circuit inhibitory neurons during critical period development in the somatosensory cortex

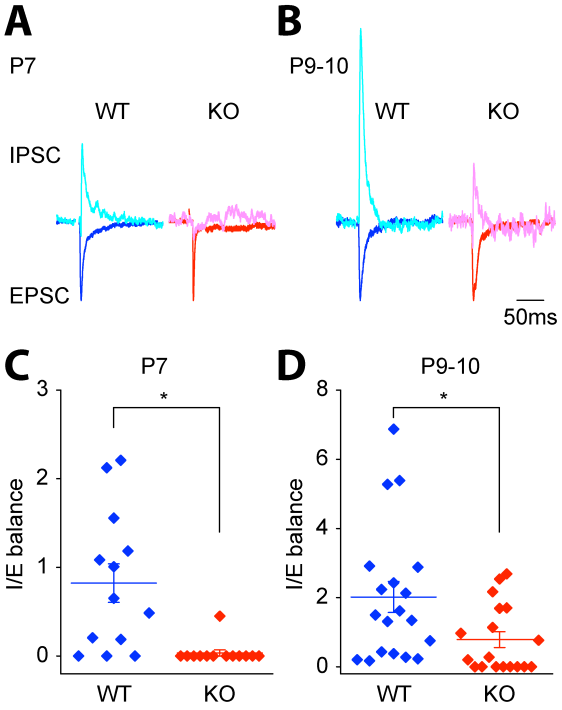

Figure 4: Excitatory and Inhibitory Input to Layer IV neurons in somatosensory cortex of Fragile X mice. A) Representative traces from Fmr1 WT and Fmr1 KO recordings. Each trace is from a single neuron showing the inward EPSC recorded at -60mV and the outward IPSC recorded at 0mV. Recordings were made from layer IV neurons in slices from mice and postnatal day 7 (P7) **B)** Representative traces of IPSCs and EPSCs from recordings from layer IV neurons in P9-10 mice. **C)** Analysis of the IPSC:EPSC amplitude for each recorded neuron in WT and KO mice at P7 and **D)** P9-10

Current work is ongoing recording from the spiny stellate neurons and also non-PV interneurons to determine if synaptic connectivity to these neuronal types is also altered. These experiments were proposed to be completed by the end of the second year of the proposal.

Subtask 2: Determine whether there are alterations in the connectivity of layer II/III neurons in Fmr1 KO mice

As we proposed in the statement of work this section would be addressed during the second year of the award. Therefore as yet there is no progress to report.

Subtask 3: Determine whether the development of extrinsic connectivity from thalamus is altered in Fmr1 KO mice

As we proposed in the statement of work this section would be addressed during the third year of the award. Therefore there is no progress to report.

Subtask 4: Determine whether the dynamic properties of individual synaptic connections in the somatosensory cortex are altered in FXS mice. This subtask will be performed between the first and second year. As yet there is no data to report.

REFERENCES

1. He, C.X. *et al.* Tactile Defensiveness and Impaired Adaptation of Neuronal Activity in the Fmr1 Knock-Out Mouse Model of Autism. *J Neurosci* **37**, 6475-6487 (2017).
2. Nomura et al Delayed Maturation of Fast-Spiking Interneuron is Rectified by Activation of the TrkB Receptor in the Mouse Model of Fragile X Syndrome. *J Neurosci* **37,** 11298-11310 (2017).

What opportunities for training and professional development has the project provided?
If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

Nothing to report

If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

Nothing to Report

What do you plan to do during the next reporting period to accomplish the goals?
If this is the final report, state "Nothing to Report."

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

We propose to continue with the data collection as it is laid out in the Statement of Work. The goals of the project have not changed and we propose to continue with the direction that we are taking. Any modifications in the scientific plan will be reported in the next period

4. **IMPACT:** Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

Nothing to report

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

Nothing to report

What was the impact on technology transfer?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Nothing to Report

What was the impact on society beyond science and technology?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

Nothing to Report

5. **CHANGES/PROBLEMS:** The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:

Changes in approach and reasons for change

Nothing to report

Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.

Actual or anticipated problems or delays and actions or plans to resolve them
Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

Nothing to report

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

N/A

Significant changes in use or care of vertebrate animals

N/A

Significant changes in use of biohazards and/or select agents

N/A

6. PRODUCTS: List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."

- **Publications, conference papers, and presentations**
  Report only the major publication(s) resulting from the work under this award.

  Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

  He CX, EA Arroyo, DA Cantu, A Goel, and C Portera-Cailliau (2018). A versatile method for viral transfection of calcium indicators in the neonatal mouse brain. *Front Neural Circuits,* in press.

  Ricard C, ED Arroyo, CX He, C Portera-Cailliau, G Lepousez, M Canepari, and D Fiole (2018) Two-photon probes for *in vivo* multicolor microscopy of the structure and signals of brain cells. *Brain Struct & Funct,* in press.

  Goel A, D Cantu, J Guilfoyle, GR Chaudhari, A Newadkar, B Todisco, D De Alba, N Kourdougli, LM Schmitt, E Pedapati, CA Erickson, and **C Portera-Cailliau**. Impaired perceptual learning in Fragile X syndrome is mediated by parvalbumin neuron dysfunction in V1 and is reversible. *Submitted* (3rd round of reviews at *Nature Neuroscience*)

  Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation);*

status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).

Nothing to report

Other publications, conference papers and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

Nothing to report

- **Website(s) or other Internet site(s)**
  List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

Nothing to report

- **Technologies or techniques**
  Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.

Nothing to report

- **Inventions, patent applications, and/or licenses**
  Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

Nothing to Report

- **Other Products**
  Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:
  - *data or databases;*
  - *physical collections;*
  - *audio or video products;*
  - *software;*
  - *models;*
  - *educational aids or curricula;*
  - *instruments or equipment;*
  - *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
  - *clinical interventions;*
  - *new business creation; and*
  - *other.*

Nothing to report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change".

Name: Anis Contractor
Project Role: PI
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: 2.4
Contribution to Project: Overall lead for the project, provides scientific direction, mentors students and postdocs, analyses data and performs administrative duties
Funding Support: None (Complete only if the funding support is provided from other than this award.)

Name: Jian Xu
Project Role: Research Assistant Professor
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: 6
Contribution to Project: Performed experiments and analyzed data
Funding Support: None (Complete only if the funding support is provided from other than this award.)

Name: Chrissy Remmers
Project Role: Graduate Student
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: 9
Contribution to Project: Performed experiments and analyzed data
Funding Support: None(Complete only if the funding support is provided from other than this award.)

Name: Yiwen Zhu
Project Role: Graduate Student
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: 12
Contribution to Project: Performed experiments and analyzed data
Funding Support: None (Complete only if the funding support is provided from other than this award.)

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

Grant R01MH099114 is now active effective 3/1/18.

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.

Provide the following information for each partnership:
Organization Name:
Location of Organization: (if foreign location list country)
Partner's contribution to the project (identify one or more)

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner's facilities for project activities);*
- *Collaboration (e.g., partner's staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and*
- *Other.*

Nothing to report

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: N/A

QUAD CHARTS: N/A

9. APPENDICES: N/A